Cell Culture Protocol

Note: Perform all procedures wearing a lab coat and gloves. Before handling sterile supplies or cell cultures, wash gloves thoroughly with 70% ethanol and do not contact anything outside of the fume hood. Prior to use, sterilize all supplies (excluding media, cells and other light-sensitive materials) in the fume hood under UV light for at least 10min. Bring all media and other solutions to 37°C in a water bath and wash bottles thoroughly with 70% ethanol prior to use in the fume hood. Remember to use sterile technique at all times (for example, do not use tips or pipets that have made contact with the external surface of tubes or plates)! When finished, thoroughly clean fume hood and aspirator with 70% ethanol.

Starting a cell culture:

1) Label petri dish with next passage number.
2) Add 10mL media (with or without antibiotics) to petri dish.
3) Bring frozen cells to 37°C quickly in water bath.
4) Add 1mL cells to media, mix by gently moving plate back and forth and side to side (do not swirl) and incubate at 37°C for 24hrs.

Changing media:

1) Use 2mL pipette and aspirator to remove used media from plate (tilt dish and remove from edge so as not to disturb cells).
2) Add 10mL of new media to plate.
3) Incubate at 37°C for two days.

Splitting cultures (1 to 3):

1) Check confluence of cells under a microscope (should be >90%).
2) Aspirate media from source culture dish.
3) Add 1mL trypsin to dish, swirl and immediately aspirate to remove residual media.
4) Add 1mL trypsin to dish, swirl and incubate at 37°C for 1min. Check that cells have become spherical under a microscope.
5) Using a pipette set to 500uL, use trypsin to rinse cells off the surface of the plate and to separate cell clumps (press tip against dish surface and slowly eject cells).
6) Add 2mL media to plate and continue to wash cells off the dish surface.
7) Add 1mL of cell culture/media/trypsin mixture to each of three new petri dishes containing 9mL of media each.
8) Incubate at 37°C overnight.

Freezing cell cultures:

1) Check confluence of cells under a microscope (should be >90%).
2) Aspirate media from source culture dish.
3) Add 1mL trypsin to dish, swirl and immediately aspirate to remove residual media.

4) Add 1mL trypsin to dish, swirl and incubate at 37°C for 1min. While waiting, label one centrifuge tube (per two plates) and add 5mL media to the tube. Label cryotubes (one per plate) with cell line (eg: HEK293), next passage number, name and date. Note: if you are freezing many cultures, you may need to label tubes prior to this step to allow adequate time. Check that cells have become spherical under a microscope.
5) Using a pipette set to 500uL, use trypsin to rinse cells off the surface of the plate and to separate cell clumps (press tip against dish surface and slowly eject cells).
6) Transfer cells from plates to centrifuge tube.
7) Centrifuge at 1500RPM for 6min at 3°C (ACC/DEC=6).
8) Prepare freezing buffer (10% DMSO in media). For example, for four cryotubes, make 5mL (4.5mL media + 0.5mL DMSO).
9) Aspirate media off of cell pellet.
10) Add 1mL freezing buffer and resuspend pellet.
11) Add another 1mL freezing buffer and mix.
12) Add 1mL of cell/freezing buffer mixture to each cryotube.
13) Freeze SLOWLY in a stryofoam chamber padded with paper towels for 1hr at -20°C.
14) Transfer to -80°C for storage.